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Changes in phenolic compounds during accelerated browning in white wines from *cv.* Pedro Ximenez and *cv.* Baladi grapes

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Changes in absorbance (A420, A280) and phenolic compounds during browning of white wines from *cv.* Pedro Ximenez and *cv.* Baladi grapes were studied. Bottled wines were stored at 50°C for 12 weeks, after which they were uncorked from the 12th to 14th week. Samples in triplicate were collected after 4, 12 and 14 weeks as well as the initial sample. A420 showed no differential susceptibility to browning for the two wines, but there were higher A280 values and phenolic compound contents in the initial samples of Pedro Ximenez wine. Caftaric and coutaric acids as well as procyanidins were the compounds showing greatest differences between the wines of the two varieties studied. Monomeric and dimeric flavan-3-ol derivatives also changed. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Browning is probably the most severe problem in white winemaking and a source of economic losses. It is well known that phenolics are the compounds of musts and wines responsible for browning. In musts, the esters of hydroxycinnamic acids play a prominent role in the process, particularly caftaric acid, which is quickly oxidized enzymatically and subsequently induces coupled oxidation reactions to other phenols leading to the formation of polymerizing quinones (Singleton *et al.*, 1984, 1985; Cheynier & Van Hulst, 1988; Cheynier *et al.*, 1989, 1990*a*,*b*; Cheynier & Ricardo da Silva, 1991; Macheix *et al.*, 1991). In finished wines, polyphenol oxidation is slower, indicating that the chemical pathway prevails over the enzymatic route. In fact, active polyphenoloxidase present in musts is rendered largely inactive by such phenomena as the deactivation by the quinones themselves, the presence of SO_2 and ethanol, precipitation during fermentation and post-fermentation stabilization and finishing processes. In red wines, anthocyanin-tannin condensation leading to the formation of polymeric pigments contributes strongly to colour stabilization (Timberlake & Bridle, 1976; Glories & Calvin, 1989; Liao *et al.*, 1992; Singleton & Trousdale, 1992), although a shift in the hue is observed during ageing. White wines contain no anthocyanins, so they lack the above-mentioned colour buffering capacity and exhibit considerably greater instability as a result of oxidation processes. With regard to this problem, flavan-3-ol monomers and oligomers have an important role in oxidation reactions (Simpson, 1982; Oszmianski *et al.*, 1985; Cheynier *et al.*, 1988; Lee & Jaworski, 1988; Cheynier & Ricardo da Silva, 1991; Dallas *et al.*, 1995), so higher contents in these compounds would lead to a greater instability of the wines to oxidation.

The Montilla-Moriles region (southern Spain) traditionally produces Sherry wines of a high alcoholic content but, lately, is also making white table wines in response to an increasing demand. These latter wines are produced from grapes of the *cv.* Pedro Ximenez type that are harvested in semiripe stage, in order not to exceed an alcohol content of 11.5% (v/v). This poses some problems arising from the need to harvest the grapes over a fairly short period of time. On the other hand, ripe grapes of *cv.* Baladi, also growing in the region, are more aromatic than Pedro Ximenez grapes and also suitable for producing white table wines. However, setting up cultures of a new grape in a winemaking region is rather costly, so a decision entails balancing such factors as expected production, agronomic management, water stress and disease resistance and others related to the quality of the resulting wines such as the browning resistance. In this work, changes in phenolic compounds during accelerated browning of white table wines from semiripe Pedro Ximenez grapes and ripe Baladi grapes were studied.

MATERIALS AND METHODS

Samples

Industrial wines obtained by fermentation of musts from semiripe grapes of *cv.* Pedro Ximenez and ripe grapes of *cv.* Baladi were treated with casein (10 g/Hl), cooled to −3°C and stabilized microbiologically by passing through filters of 0.45 μm pore size (Millipore, Water Associates). Subsequently, the wines were bottled and stoppered with 45 mm long corks.

Three batches of bottled wine of each variety were selected for triplicate experiments. Each batch consisted of four bottles from which samples were collected at 0 (initial sample), 4, 12 and 14 weeks. Except for the initial sample, all other bottles were placed in a thermostated oven at 50°C, from which they were removed at the above-mentioned times. In order to emphasize the differences in browning between the wines from the two varieties studied, the bottles were uncorked from the 12th to 14th week. Therefore, the samples collected up to the 12th week evolved under anaerobic conditions, whereas that taken at the 14th week remained under aerobic conditions for the last two weeks. The volume lost by evaporation in this last period was measured in order to correct the results.

Analytical procedures

Ethanol was quantified by the Crowell & Ough (1979) method. The levels obtained (% v/v) for the Pedro Ximenez and Baladi wines were 10.7 ± 0.06 and 10.1 ± 0.11, respectively. Spectrophotometric measurements were made at 420 and 280 nm in a Perkin–Elmer spectrophotometer, Lambda 3 model, on 10 mm pathlength.

Extraction

A volume of 100 ml of wine was concentrated in vacuum at about 40°C up to 20 ml, which was adjusted to pH 7 with 0.1 N NaOH. The concentrate was passed through a Sep–Pak cartridge (Water Associates) that was previously activated with methanol and adjusted to pH 7 with NaOH according to Jaworski & Lee (1987). For collection of the phenolic acid fraction, the cartridge was eluted with water at pH 7. After preconditioning of the cartridge with 2 ml of water at pH 2, the flavan-3-ol fraction was eluted with 16% acetonitrile in water at pH 2 (Oszmianski *et al.*, 1988). The two collected fractions were concentrated and passed through a filter of 0.45 μm pore size for injection into a Spectra–Physics SP880 HPLC instrument.

Identification

The identification of the phenolic compounds was achieved by comparing with the retention times of the standards, UV spectra obtained by an HPLC Rapid Scanning Detector (Spectra–Physics mod. Focus) and calculation of UV absorbance ratios after coinjection of samples and standards (Mayen *et al.*, 1995). Commercial standards were purchased from Sigma–Aldrich Chem. Co. (Madrid, Spain) and Sarsynthese Co. (Genay, France). Caftaric and Coutaric acids were isolated by the method described by Singleton *et al.* (1978). Procyanidins were obtained from a grape seed extract according to Bourzeix *et al.* (1986). The standards purity was 95–99%. The quantified compounds in each fraction were:

- Phenolic acids fraction:
  - (a) Hydroxybenzoic acids—gallic, protocatechuic, m-hydroxybenzoic, vanillic and syringic.
  - (b) Hydroxycinnamic acids—caffeic, p-coumaric and ferulic.
  - (c) Esters of hydroxycinnamic acids—caftaric, coutaric and feftaric
- Flavan-3-ol fraction:
  - (a) Catechins—catechin and epicatechin.
  - (b) Procyanidins—B1, B2, B3 and B4.

Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except the procyanidins that were quantified as catechin.

HPLC analyses

The chromatographic conditions employed were as follows:
C18 column (250×4.6 mm, 5 μm size particle).
Mobile phase: 2% acetic acid and acetonitrile.

	Acids Ph. fraction	Flavan-3-ol fraction
Flow-rate	2 ml/min	2 ml/min
Wavelength	280 nm	280 nm
Elution	$\%CH_3CN$	$\%CH_3CN$
0 min	0.1	0.1
5 min	5	
15 min	5	15
20 min	15	15
25 min		20
30 min	15	30
40 min	100	100

Statistical procedures

Principal component analyses were performed on the replicated samples by using a Statgraphics Statistical Computer Package (Statistical Graphics Corp.).

RESULTS AND DISCUSSION

Figure 1 shows the total polyphenol contents obtained as absorbance values at 280 nm. As can be seen, the

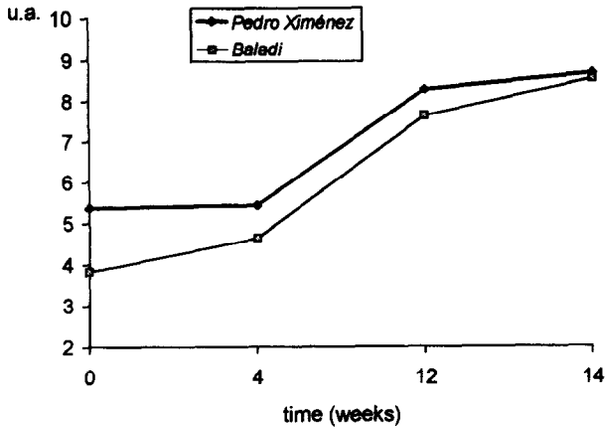

Fig. 1. Total phenolic compounds measured as absorbance at 280 nm.

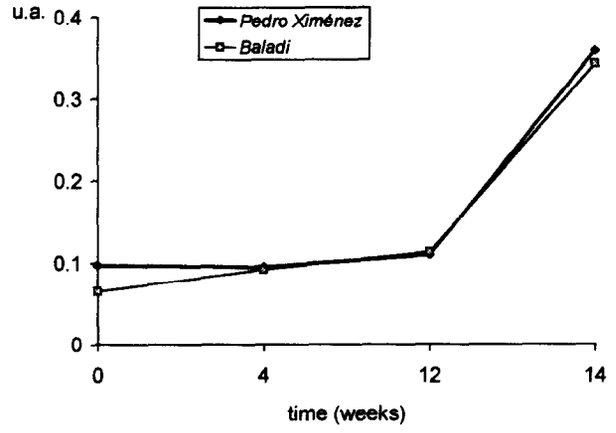

Fig. 2. Browning measured as absorbance at 420 nm.

absorbance increased throughout the period studied for the wines of both grapes varieties. At the beginning, the absorbance of the Pedro Ximenez wine exceeded that of the Baladi wine by about 40%. Later the difference gradually decreased and virtually cancelled during the period where the bottles were uncorked (between the 12th and 14th week).

There were no appreciable differences in absorbance values at 420 nm between the two wines (Fig. 2), nor did browning at this wavelength increase significantly until the 12th week, when the bottles were uncorked. Obviously, the absorbance at 420 nm increased markedly (by more than 200%) on contact of the wines with the atmosphere (from 12th to 14th week), with no appreciable differences between the two wines in this respect.

Figures 3 and 4 show the chromatograms obtained for both fractions of phenolic compounds determined and Table 1 lists their contents. Overall, the fraction containing the esters of hydroxycinnamic acids (caftaric, coutaric and feftaric) was the most abundant in both wines (particularly in Pedro Ximenez), followed by that of flavan-3-ol monomers and dimers, the latter prevailing among the procyanidins, the contents of which increased during the time the bottles were kept corked and decreased afterwards (12th–14th week). It is worth noting the sharp increase in the content of an unidentified compound (named peak 3), which became one of the major constituents by the end of the experiment. This compound exhibited maximum UV–V absorption at 284 nm. Based on the fact that peak 3 was increased with browning, it may have been given as a result of some phenol oxidation product (possibly a semiquinone or hydroquinone).

In order to identify changes in the contents of the different phenolic compounds during browning, the results were subjected to a multivariate analysis of principal components. For the Pedro Ximenez wines, Fig. 5 shows the representation of the results obtained for each triplicate sample on the plane defined by the first two components, in addition to the vectors reflecting the contributions of the different phenols to each component. The two components accounted for 95.1% of the overall variance. As can be seen, the most prominent variables in terms of weight were peak 3 and procyanidins, followed by caftaric acid, coutaric acid, catechin, peak 2 and epicatechin; all other variables

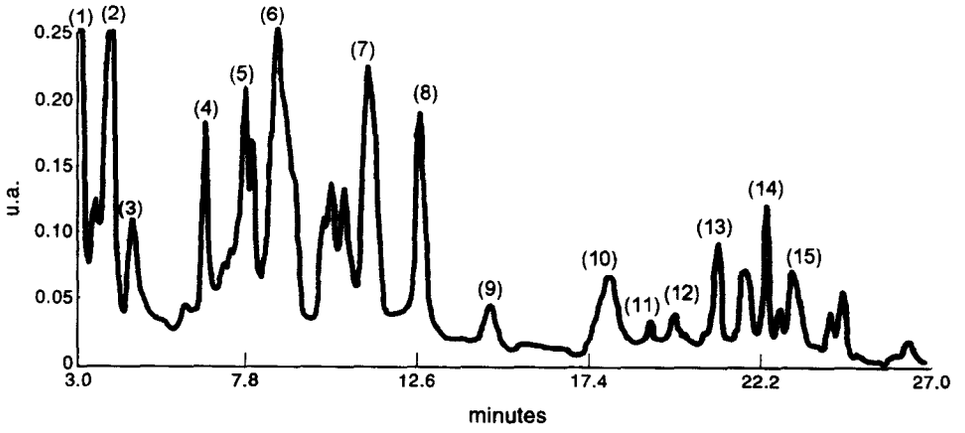

Fig. 3. Chromatogram of phenolic acids fraction: (1)-peak 1, (2)-gallic, (3)-peak 2, (4)-peak 3, (5)-protocatechuic, (6)-t-caftaric, (7)-c-coutaric, (8)-t-coutaric, (9)-m-hydroxybenzoic, (10)-feftaric, (11)-vanillic, (12)-caffeic, (13)-syringic, (14)-p-coumaric and (15)-ferulic.

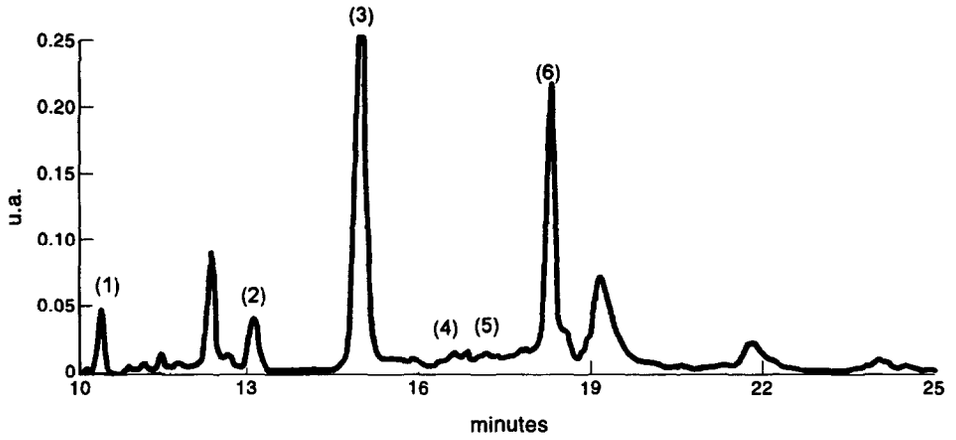

Fig. 4. Chromatogram of flavan-3-ol fraction: (1)-procyanidin B1, (2)-procyanidin B3, (3)-catechin (4)-procyanidin B4, (5)-procyanidin B2 and (6)-epicatechin.

Table 1. Phenolic compound contents during browning of wines (mg/l)

Wines	Compounds	Time (weeks)			
		0	4	12	14
Pedro Ximenez	Gallic	5.08 ± 1.05	6.28 ± 0.414	7.56 ± 1.30	6.42 ± 0.012
	Protocatechuic	0.530 ± 0.143	1.64 ± 0.287	1.61 ± 0.029	0.755 ± 0.226
	m-Hydroxybenzoic	0.568 ± 0.017	0.599 ± 0.094	0.669 ± 0.023	1.12 ± 0.316
	Vanillic	0.102 ± 0.024	0.156 ± 0.038	0.115 ± 0.003	0.219 ± 0.054
	Syringic	0.283 ± 0.031	0.245 ± 0.005	0.304 ± 0.067	0.315 ± 0.041
	Caffeic	0.974 ± 0.213	1.69 ± 0.345	3.26 ± 0.339	3.09 ± 0.007
	p-Coumaric	0.282 ± 0.021	0.065 ± 0.021	0.243 ± 0.060	0.130 ± 0.005
	Ferulic	0.442 ± 0.129	0.765 ± 0.129	2.12 ± 0.210	2.41 ± 0.074
	Caftaric	20.7 ± 1.77	20.1 ± 1.43	26.6 ± 2.54	21.7 ± 0.650
	Coutaric	18.0 ± 1.61	18.9 ± 0.661	15.8 ± 0.503	19.2 ± 0.100
	Feftaric	2.33 ± 0.277	5.36 ± 0.483	5.37 ± 0.594	5.62 ± 0.204
	Procyanidins	29.0 ± 1.02	29.3 ± 0.321	40.3 ± 1.88	28.4 ± 0.700
	Catechin	6.76 ± 0.530	6.80 ± 0.566	9.32 ± 1.42	10.2 ± 0.173
	Epicatechin	7.15 ± 0.255	7.25 ± 0.358	10.4 ± 0.376	8.99 ± 0.650
	Peak 1	1.60 ± 0.204	2.77 ± 0.240	1.84 ± 0.243	1.92 ± 0.186
	Peak 2	1.05 ± 0.059	1.80 ± 0.389	4.73 ± 0.975	2.71 ± 0.053
	Peak 3	0.808 ± 0.031	2.30 ± 0.225	10.3 ± 0.796	18.4 ± 1.68
Baladi	Gallic	4.59 ± 0.495	4.99 ± 0.657	5.31 ± 0.509	5.66 ± 0.642
	Protocatechuic	1.09 ± 0.178	1.25 ± 0.250	1.02 ± 0.286	1.78 ± 0.380
	m-Hydroxybenzoic	0.318 ± 0.053	0.776 ± 0.160	0.422 ± 0.008	< 0.001
	Vanillic	0.151 ± 0.016	0.514 ± 0.011	0.053 ± 0.002	< 0.001
	Syringic	0.241 ± 0.013	0.413 ± 0.018	0.294 ± 0.080	< 0.001
	Caffeic	0.767 ± 0.116	1.13 ± 0.288	2.07 ± 0.054	1.94 ± 0.324
	p-Coumaric	0.407 ± 0.073	< 0.001	0.053 ± 0.007	< 0.001
	Ferulic	0.161 ± 0.018	0.456 ± 0.072	0.384 ± 0.059	0.244 ± 0.019
	Caftaric	14.5 ± 0.900	16.2 ± 1.2	16.1 ± 0.200	15.3 ± 2.85
	Coutaric	12.6 ± 0.451	10.9 ± 0.665	11.5 ± 0.945	14.2 ± 0.152
	Feftaric	6.51 ± 0.263	5.19 ± 0.737	4.56 ± 0.340	4.63 ± 0.047
	Procyanidins	19.5 ± 1.2	32.8 ± 1.83	35.9 ± 0.902	33.7 ± 0.854
	Catechin	10.3 ± 0.380	9.40 ± 0.774	9.83 ± 0.479	8.25 ± 0.386
	Epicatechin	5.08 ± 0.335	6.77 ± 0.420	10.8 ± 1.16	10.5 ± 0.640
	Peak 1	3.30 ± 0.085	3.77 ± 0.535	4.07 ± 0.776	3.95 ± 0.646
	Peak 2	2.08 ± 0.231	2.06 ± 0.071	5.48 ± 1.12	3.77 ± 0.490
	Peak 3	2.04 ± 0.125	4.23 ± 0.070	14.4 ± 0.435	22.5 ± 0.907

contributed very little by contrast. Some authors such as Simpson (1982) have reported that the procyanidins and catechins contents have a strong influence on the susceptibility of white wines to browning.

During the first period of the study (0 and 4th week), samples exhibited small differences in relation to component 1, which was essentially influenced by peak 3 and no difference in terms of component 2. On the other hand, the samples measured at the 12th week and the end of the experiment (14th week) behaved similarly in relation to component 1 but they were separated by component 2, which was markedly affected by procyanidins.

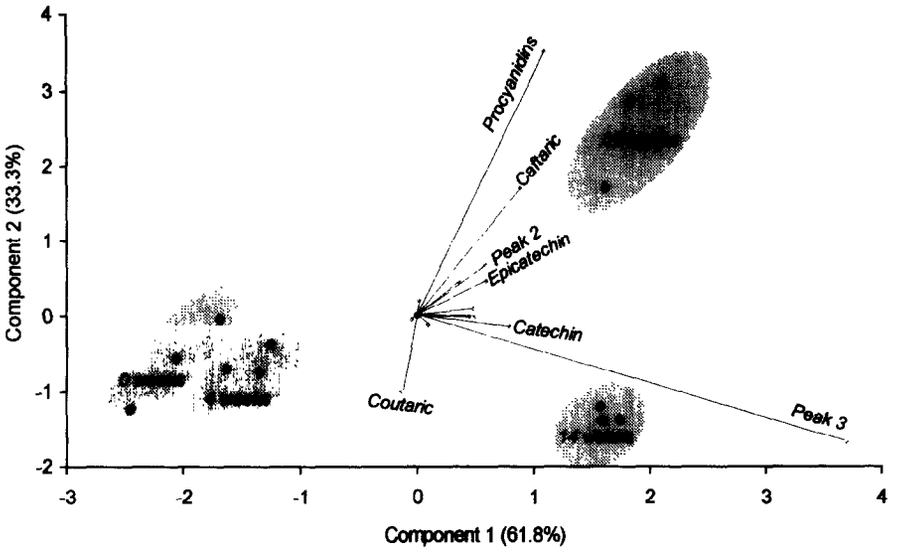

Fig. 5. Principal component analysis. Biplot representation of samples and variables for Pedro Ximenez wines.

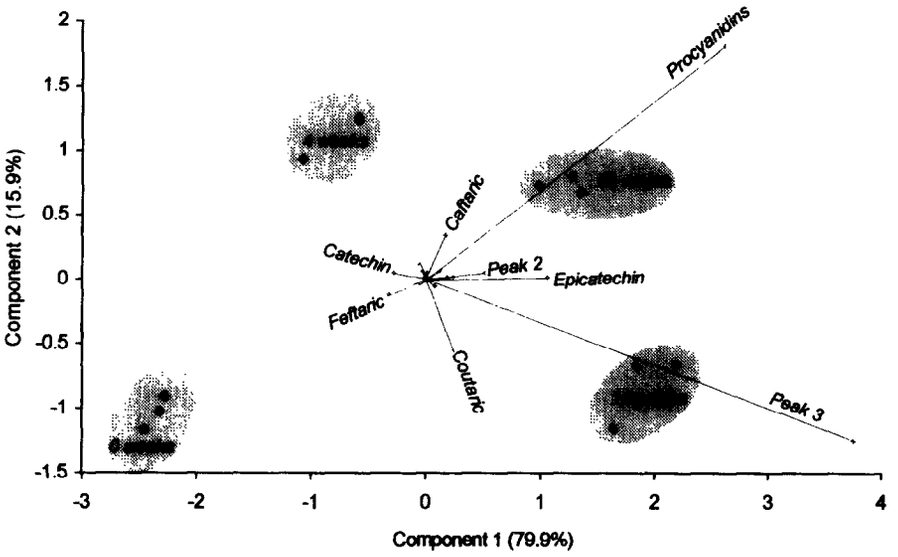

Fig. 6. Principal component analysis. Biplot representation of samples and variables for Baladi wines.

Basically, the behaviour of Baladi wines (Fig. 6) was similar to that of Pedro Ximenez samples as regards the most influential variables (peak 3 and procyanidins). However, some differences were observed in the samples measured at the 4th week, which in Baladi wines were separated from the initial values as a result of the increase in the procyanidin contents and peak 3.

In order to compare the behaviour of the wines of the two grape varieties, the results were subjected to a multivariate analysis of principal components. As can be seen in Fig. 7, the first two components accounted for 80.5% of the overall variance. Component 1 induced a more marked separation between samples throughout the browning process, whereas component 2 separated the wines from the two grape varieties studied. Essentially, the separation was established by the caftaric and coutaric acid contents, which exhibited a substantial weight on component 2 but not on component 1. Specifically, the wine samples from Pedro Ximenez grapes exhibited higher concentrations of these acids than those from Baladi grapes. Procyanidins and peak 3 had a substantial weight in both components, so they had a similar resolving effect on components 1 and 2 (between varieties and browning stages).

Overall, the higher A280 values and phenol contents in the initial samples of wines from Pedro Ximenez grape variety (95.65 mg/L for Pedro Ximenez and 83.63 mg/L for Baladi) did not result in a differential susceptibility to browning, measured as absorbance at 420 nm. Therefore, both types of wine should behave similarly in this respect under real marketing conditions (lower temperatures than in this experiment).

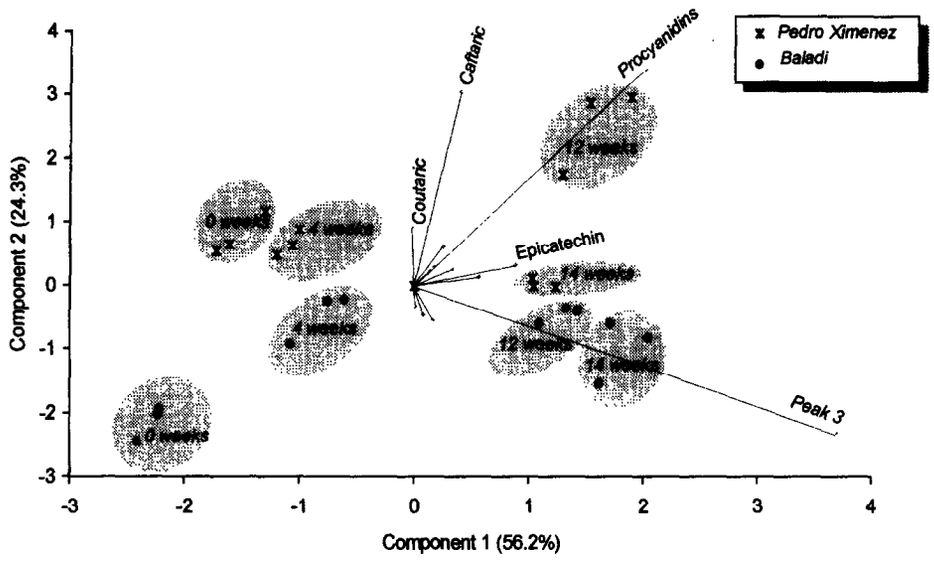

Fig. 7. Principal component analysis. Biplot representation of samples and variables for Pedro Ximenez and Baladi wines.

On the other hand, accelerated browning in the wines from the two grape varieties in the absence of contact with atmospheric oxygen mainly increased epicatechin and procyanidin contents and also peak 3. The increased contents of monomeric and dimeric flavan-3-ol derivatives can be reasonably attributed to hydrolysis of oligomeric derivatives, favoured by the experimental temperature. These hydrolytic reactions have been suggested by some authors for the C1 and T2 trimers and galloylated dimers (Timberlake & Bridle, 1976; Dallas *et al.*, 1995). Concomitant with this potential hydrolysis, these compounds would have been oxidized to a moderate extent during the anaerobic period of the experiment, which may have been the reason for the increase in peak 3 during the first 12 weeks. The aerobic period (12th–14th weeks) accelerated the oxidation markedly and led to a stabilization and/or decreasing in flavan-3-ol monomer and dimer contents and a markedly increased peak 3. In fact, peak 3 increased as much during the last two weeks as in the previous 12 weeks under anaerobic conditions. Overall, if the hypothesis of concomitance of hydrolytic and oxidative processes is accepted for these compounds, during the anaerobic period the first prevails and during the aerobic the last.

As regards the other polyphenols studied, their contents remained fairly stable. In particular, hydroxycinnamic esters varied very little during the browning experiment. Caftaric acid is known to be the substrate responsible for the initiation of enzymatic browning in must, however, it does not seem to have such a marked effect as flavan-3-ol derivatives on chemical browning in white wines.

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